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Determination of Free Amino Compounds in Betalainic Fruits and Vegetables by Gas Chromatography with Flame Ionization and Mass Spectrometric Detection

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Amino acids and amines are the precursors of betalains. Therefore, the profiles of free amino compounds in juices obtained from cactus pears [*Opuntia ficus-indica* (L.) Mill. cv. Bianca, cv. Gialla, and cv. Rossa], pitaya fruits [*Selenicereus megalanthus* (K. Schumann ex Vaupel) Moran, *Hylocereus polyrhizus* (Weber) Britton & Rose, and *Hylocereus undatus* (Haworth) Britton & Rose], and in extracts from differently colored Swiss chard [*Beta vulgaris* L. ssp. *cicla* (L.) Alef. cv. Bright Lights] petioles and red and yellow beets (*B. vulgaris* L. ssp. *vulgaris* var. *conditiva* Alef. cv. Burpee's Golden) were investigated for the first time. Amino compounds were derivatized with propyl chloroformate. While gas chromatography (GC) with mass spectrometry was used for peak assignment, GC flame ionization detection was applied for quantification of individual compounds. Whereas proline was the major free amino compound of cactus pear and pitaya fruit juices, glutamine dominated in Swiss chard stems and beets, respectively. Interestingly, extremely high concentrations of dopamine were detected in Swiss chard stems and beets. Furthermore, the cleavage of betaxanthins caused by derivatization in alkaline reaction solutions is demonstrated for the first time. Amino acids and amines thus released might increase the actual free amino compound contents of the respective sample. To evaluate the contribution of betaxanthin cleavage to total amino acid and amine concentration, isolated betaxanthins were derivatized according to the "EZ:faast" method prior to quantification of the respective amino compounds released. On a molar basis, betaxanthin contribution to overall amino compound contents was always below 6.4%.

KEYWORDS: ***Beta vulgaris*; *Opuntia*; *Hylocereus*; *Selenicereus*; betaxanthins; amino acids; amines; gas chromatography**

INTRODUCTION

In contrast to the widespread anthocyanins suitable for low pH products, betalains are only present in 13 families of the plant order Caryophyllales (*1*) and some genera of the Basidiomycetes (*2*). Up to now, mainly red beet preparations are used to color low-acid foodstuffs. Because of the growing demand for coloring foodstuffs (*3*), the search for new colored plant materials including the characterization of their composition is stimulated. The exact knowledge of amino compound composition is important for the nutritional evaluation of the respective foodstuff but also for monitoring changes upon heat processing. Furthermore, in the case of betalainic fruits and vegetables, the knowledge of amino compound composition is a prerequisite for better understanding betalain biosynthesis, because amines and amino acids together with betalamic acid are betalain precursors. Therefore, the present study aimed at a detailed study of amino compound composition of betalainic foodstuffs such as yellow and red beet, differently colored and colorless Swiss chard petioles, and juices from cactus pears and pitaya fruits applying both gas chromatography flame ionization (GC-FID) and mass spectrometric (MS) detection.

Conventional derivatization methods hitherto applied for amino acid analysis include the use of reaction solutions typically buffered in the pH range of pH 8.5−10 (*4*). Sometimes, even heating is necessary for complete reaction. Therefore, a release of amino compounds from betaxanthins (bxs) present in the derivatized sample must be taken into account. *ortho*-Phthaldialdehyde derivatization is performed at a pH of around 10 (*4*, *5*). Derivatization with phenylisothiocyanate requires the addition of triethylamine and tedious drying steps (*6*), which might also lead to bx degradation and concomitant release of amino compounds (*7*). Dabsyl chloride reaction with amino

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compounds requires a pH of 8.6 and incubation at 70 °C for 15 min (*8*), possibly cleaving bxs into betalamic acid and the corresponding amino compound. For derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate previously applied for amino acid profiling of bx-containing hairy roots from *Beta vulgaris* var. *lutea* (*9*), the reaction medium is buffered to pH 8.8 and heated for 10 min at 55 °C (*10*). Whereas dopamine, 3-methoxytyramine, and tryptophan can be detected by their inherent fluorescence and ultraviolet absorption (*11*), most amino compounds need derivatization for high-performance liquid chromatography (HPLC) or GC detection. Because bxs and amino compounds display similar physicochemical properties, complete separation of the latter from its potential precursor appears to be difficult. Therefore, it has to be taken into account that derivatization of amino compounds being released from bxs might result in overestimation of the actual amino compound concentrations of the particular food.

Despite requiring alkaline conditions, the "EZ:faast" method using propyl chloroformate as a derivatization reagent was considered suitable for the present investigation due to easy sample workup, fast precolumn derivatization, and short GC analyses. In addition, both primary and secondary amino compounds can be assessed. Therefore, in this study, it should be examined whether bx cleavage caused by the alkaline derivatization procedure contributes to the release of amino compounds thus increasing their apparent contents in the particular plant tissue studied. Amino compound analysis in the presence of betalains, especially bxs, presents an analytical challenge, which to the best of our knowledge has not been considered so far.

MATERIALS AND METHODS

Plant Material. Differently colored Swiss chard [*B. vulgaris* L. ssp. *cicla* (L.) Alef. cv. Bright Lights, Chenopodiaceae] with purple, red-purple, yellow-orange, yellow, and white petioles was purchased from Pommerenke (Steinheim am Albuch, Germany). It was harvested at the end of September 2004, 13 weeks after sowing. After washing, the leaves were removed and the petioles were frozen in liquid nitrogen and sealed in polyacrylamide−polyethylene bags under reduced pressure for storage at −80 °C until further processing.

Seeds of yellow beet (*B. vulgaris* L. ssp. *vulgaris* var. *conditiva* Alef. cv. Burpee's Golden, Chenopodiaceae) were sown in May 2004 in the Experimental Station for Horticulture of Hohenheim University, and fully developed hypocotyls were harvested in July 2004. Red beet (*B. vulgaris* L. ssp. *vulgaris* var. *conditiva* Alef., Chenopodiaceae) was purchased in October 2004 at a local market. After the beets were washed and the hair roots were removed, yellow beets were cut by cross-section and only the bottom half with regular pigment distribution in the peel was further used. Whereas yellow beet material was immediately comminuted by cryogenic grinding (see below), red beets were cut in slices, frozen in liquid nitrogen, sealed in bags under reduced pressure, and finally stored at −80 °C until further sample preparation.

Cactus pear cultivars [*Opuntia ficus-indica* (L.) Mill. cvs. Bianca, Gialla, and Rossa, Cactaceae] were imported from Sicily (Italy) in October 2004. In November 2004, *Hylocereus polyrhizus* (Weber) Britton & Rose (Cactaceae) with purple pulp and *Hylocereus undatus* (Haworth) Britton & Rose (Cactaceae) with white pulp were obtained from Israel while *Selenicereus megalanthus* (K. Schumann ex Vaupel) Moran (Cactaceae) with yellow peel and white flesh was imported from Colombia.

Solvents and Reagents. Reagents and solvents were purchased from VWR (Darmstadt, Germany) and were of analytical or HPLC grade. Deionized water was used throughout.

Cryogenic Grinding of Swiss Chard Petioles and Beet Roots. As described earlier (*12*), comminution of Swiss chard petioles and beet roots was performed in a Waring blender model 38BL41 (Waring Products, Torrington, CT). To preclude enzymatic action during grinding, liquid nitrogen was added. The resulting powder was kept at −80 °C until extraction.

Extraction of Swiss Chard and Beet Powder. Amino compounds and pigments, respectively, were extracted from 150 g of Swiss chard powder and 100 g of beet powder with precooled 60% aqueous methanol (v/v) to inhibit residual enzyme activity (*12*, *13*). A 50 mM concentration of sodium ascorbate was added as an antioxidant. For extraction of Swiss chard, 600 mL of solvent was used, while 300 and 600 mL were applied for yellow and red beet powder, respectively. After it was stirred for 20 min at 4 °C, the plant material was separated from the extract by filtering through a glass frit under reduced pressure. For complete extraction, the residue was rinsed with 100 mL of solvent and finally with 100 mL of methanol. Red beet residues were re-extracted with 200 mL of methanol saturated with sodium ascorbate for 10 min at 4 °C and then filtered through a glass frit. Extracts were concentrated in vacuo at 30 °C, resuspended in 50 mL of purified water, flushed with nitrogen, and stored at −80 °C. For GC and HPLC analyses as well as photometric quantification, extracts were membrane-filtered (0.45 μm; Acrodisc Premium Filter, Pall, Ann Arbor, MI).

Extraction of Cactus Fruits. *O. ficus-indica* fruits were cut in half and manually squeezed, and the resulting juice was filtered through a paper filter (604 A 1/2, Schleicher & Schuell, Dassel, Germany). To 300 mL of precooled 60% aqueous methanol (v/v) containing 50 mM sodium ascorbate, about 100 g of juice was added to reach a final volume of 400 mL. Juice from pitaya fruits was extracted the same way except for filtering the juice through a fine-mesh sieve (180 μm). The cactus fruit extracts were concentrated in vacuo at 30 °C, resuspended in 50 mL of purified water, and treated as described for Swiss chard and beet extracts, respectively.

Sample Preparation and Derivatization Procedure for GC Analysis of Amino Compounds. Purification of extracts and derivatization of amino compounds were performed applying the "EZ:faast" GC-FID kit for free amino acid analysis from Phenomenex (Torrance, CA) according to the instructions provided by the manufacturer. Tips containing sorbents for solid phase extraction (SPE), all reagents required for SPE and derivatization, and amino acid standard solutions (200 μM) were supplied in this kit. Because of the presence of *allo*-isoleucine in one standard mixture solution, complete baseline separation for leucine/isoleucine was not accomplished. In addition, distinct baseline separation for aspartic acid and methionine could not be achieved. Therefore, standard solutions of the respective amino acids as well as of amino compounds not included in kit standard mixtures such as γ-amino-*n*-butyric acid, tyramine, 3-methoxytyramine, dopamine, and 3,4-dihydroxyphenylalanine were prepared separately in purified water (400 μM) and used for compound identification as well as for calibration. Additionally required standard amino compounds were purchased from Fluka (Buchs, Switzerland) and Sigma-Aldrich Co. (St. Louis, MO), respectively, and were of analytical grade.

For purification and derivatization, 100 μL of appropriately diluted extract or standard solution and 100 μL of internal standard (200 μM norvaline in 0.01 M HCl) were pipetted into a glass vial included in the "EZ:faast" kit and passed through a SPE tip. Under acidic conditions, amino compounds were bound to the sorbent material by cation exchange. Following washing with 200 μL of 2-propanol/water, amino compounds were eluted with 200 μL of alkaline eluting medium consisting of sodium hydroxide in water, 2-propanol, and an organic base. The eluate was mixed thoroughly with 50 μL of an organic solution containing propyl chloroformate as the derivatization reagent by vortexing twice for 10 s. After each vortexing, the reaction was allowed to proceed for 70 s. Derivates were then extracted by addition of a mixture of chloroform and isooctane. For GC-FID analysis, the organic layer containing amino compound derivates was further purified with 0.1 M HCl prior to injection. Only freshly derivatized samples were used for quantification of amino compounds by GC-FID. Quantitative determinations were performed in triplicate.

For GC-MS analysis, the organic layer containing derivates was transferred into a GC vial and removed with a gentle stream of nitrogen. The residue was redissolved in a mixture of isooctane and chloroform (80/20, v/v) and analyzed by GC-MS. Samples derivatized for

identification of amino compounds by GC-MS were stored not longer than 24 h at −80 °C until analysis.

All extracts were checked for the presence of genuine norvaline, which was used as an internal standard for the quantification of amino compounds in this study. For this purpose, a mixture of 100 μL of diluted extract with 100 μL of 0.01 M HCl (without norvaline) was derivatized and analyzed by GC-MS.

GC Determination of Amino Compounds. A Zebron ZB-AAA column (10 m × 0.25 mm; Phenomenex) was used for both GC-FID and GC-MS detection.

GC-FID Conditions. A CP 9001 gas chromatograph equipped with a CP 9010 autosampler and flame ionization detector, controlled by Maestro 2.4 software, was used (Chrompack, Middelburg, Netherlands). The flow rate of the carrier gas (He) was kept at 1.7 mL/min (measured at 110 °C). Hydrogen, synthetic air, and nitrogen were used as auxiliary gases for the FID detector.

The oven temperature program was as follows: Starting at 110 °C, the oven was heated to 294 °C (25 °C/min), held for 0.64 min at 294 °C, and then ramped to 320 °C at 25 °C/min with a holding time of 1 min. The temperature of the injection port was 250 °C while the detector was kept at 320 °C. Sample volumes of 2 μL were injected in split mode (1:12.9, v/v).

GC-MS Conditions. Identification of amino compounds was carried out on a TRACE GC 2000 gas chromatograph (Thermo Electron, Waltham, MA) equipped with a PAL autosampler (CTC Analytics, Zwingen, Switzerland) and coupled to a Polaris Q ion trap mass spectrometer (Thermo Electron). Xcalibur Version 1.4 SR1 software was used for acquisition and analysis of mass spectra. Helium was used as the carrier gas at a flow rate of 1.1 mL/min.

Starting at 110 °C, the oven temperature was increased to 320 °C (15 °C/min) and finally held at 320 °C for 5 min. The ion trap mass spectrometer was operated in positive electron ionization mode at an ionization energy of 70 eV. The temperatures of the injector, the ion source, and the transfer line were set at 250, 200, and 300 °C, respectively. Ten microliters was injected in split mode (1:15.5, v/v). Full scans were recorded over a mass range from 50 to 500 u, and identification of amino compounds was carried out by comparison of retention times and mass spectra with those of derivatized standard compounds.

Identification and Quantitative Determination of Betalains. Identification and quantitative betalain determination were performed on a series 1100 HPLC (Agilent, Waldbronn, Germany) connected in series with a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer and a UV−vis spectrometer (Perkin-Elmer, Überlingen, Germany) equipped with UVWinLab V 2.85.04 software (Perkin-Elmer Instruments, Norwalk, CT) as described previously (*12*). To improve peak resolution, HPLC runs were performed at 30 °C instead of 25 °C.

For photometric quantification of betalains, the aqueous pigment extracts were diluted with McIlvaine buffer (pH 6.0, citrate-phosphate) to obtain absorption values of $0.8 \leq A \leq 1.2$ at their respective absorption maxima. Measurements were performed in triplicate, and the betalain content (BC) was calculated according to ref *14*: $BC\ (mg/L) = [(A \times DF \times MW \times 1000/\epsilon \times L)]$ where A is the absorption value at the absorption maximum corrected by the absorption at 600 nm, DF is the dilution factor, and L is the path length (1 cm) of the cuvette. For quantification of betacyanins, the molecular weight (MW) and molar extinction coefficient (ϵ) of betanin (MW = 550 g/mol; ϵ = 60000 L/mol cm in H_2O; *12*) were applied. Quantitative equivalents of the major bxs were determined spectrophotometrically by applying a mean molar extinction coefficient (ϵ = 48000 L/mol cm in H_2O; *12*) and the molecular weight of glutamine-bx (vulgaxanthin I; MW = 339 g/mol) for the extracts from yellow Swiss chard petioles and yellow beet, and the molecular weight of proline-bx (indicaxanthin; MW = 308 g/mol) for Gialla juice, respectively. In the case of Gialla juice exhibiting both betacyanins (535 nm) and bxs (470 nm), additional correction of the betanin and proline-bx equivalents by the respective area proportions obtained by duplicate HPLC determination was carried out (*14*) to achieve more precise bx values. Then, individual contents of glutamine-bx, proline-bx, and dopamine-bx (miraxanthin V) monitored by HPLC analyses were calculated applying the following equation considering a molecular weight correction factor (*15*):

$$Bx_{indiv}\,(\mu mol/L) = Bx_{equivalents}\,(\mu mol/L) \times A(Bx_{indiv})/A(Bx_{total}) \times MW(Bx_{equivalents})/MW(Bx_{indiv})$$

where A is the peak area at 470 nm and MW the specific molecular weight, respectively. For dopamine-bx, a molecular weight of 346 g/mol was applied.

Isolation of Indicaxanthin, Miraxanthin V, and Vulgaxanthin I. *Indicaxanthin and Miraxanthin V.* Indicaxanthin was isolated from juice of *O. ficus-indica* cv. Gialla, and miraxanthin V was obtained from extracts of yellow Swiss chard petioles. To precipitate pectic substances, 100 mL of 2-propanol was added to 50 mL of Gialla juice and aqueous Swiss chard extract, respectively. Precipitates were removed after 15 min by filtration through a filter paper 595 A1/2 (Schleicher & Schuell, Dassel, Germany), and the filtrate was concentrated in vacuo at 30 °C to about 10 mL.

Ten milliliters of the depectinized pigment extracts was applied to an XAD-16 HP-column (830 mm × 40 mm i.d.) conditioned with 3 L of water acidified with trifluoroacetic acid (TFA, pH 3). Sugars were removed from the samples by rinsing with 17 L of acidified water (TFA, pH 3). Then, the bxs were eluted with 1.2 L of acidified methanol [95/5, methanol/acidified water (TFA, pH 2), v/v]. The collected bx eluate was adjusted to pH 5 with 4% NH_4OH to prevent acid bx hydrolysis, concentrated in vacuo at 30 °C, and used for semipreparative isolation.

Semipreparative isolation was carried out on an HPLC system (Bischoff, Leonberg, Germany) consisting of an LC-CaDI 22-14 control unit, two HPLC compact pumps connected with a UV−vis detector SPD 10 AV VP (Shimadzu, Tokyo, Japan), and a dynamic mixing chamber (Knauer, Berlin, Germany) equipped with Bischoff McDAcq 32 software. Separation was achieved on a semipreparative C_{18} AQUA column (250 mm × 21.2 mm i.d., 5 μm, Phenomenex) operated at room temperature, at a flow rate of 9 mL/min and a pressure of 70 bar. The mobile phase consisted of 0.5% formic acid in water (v/v, eluent A) and a mixture of MeCN in water of 50/50 (v/v, eluent B).

Indicaxanthin was isolated from depectinized and desalted cactus juice starting with 84% of eluent A in B, followed by a linear gradient to 72% A in B at 14 min, and then to 0% A in B in 2 min, held for 2 min before re-equilibration to starting conditions. Miraxanthin V was isolated from depectinized and desalted Swiss chard extract applying the same gradient program as used for the isolation of indicaxanthin except for the prolonged holding time (5 min) from 72% A in B to 0% A in B.

Aliquots of 700 μL of the respective pigment extracts were injected, and monitoring was performed at 470 nm for indicaxanthin (R_t = 16.0 min) and miraxanthin V (R_t = 22.0 min), respectively. The collected effluents were immediately cooled in an ice bath and gently concentrated under reduced pressure at 30 °C. Residual formic acid was removed by repeated addition of purified water and concentration. After lyophilization of the indicaxanthin solution, the resulting powder was stored at −26 °C until further analysis.

In the case of miraxanthin V, removal of colorless phenolics and pigments was accomplished by applying the aqueous fraction to a Sephadex LH-20 column (650 mm × 30 mm i.d.) preconditioned with deionized water. Pigment elution was performed with deionized water. Further purification was achieved by twice repeated semipreparative HPLC starting with 92% A in B, followed by a linear gradient to 86% A in B at 14 min and then to 77% A in B at 16 min. After a holding time of 7 min, reequilibration to starting conditions was carried out. For distinction between phenolic compounds and the almost coeluting miraxanthin V (R_t = 22.0 min), monitoring was performed at 280 nm. After evaporation under reduced pressure at 30 °C, the aqueous miraxanthin V solution was once more applied to the Sephadex LH-20 column as described above. HPLC-diode array detection (DAD) revealed that the purity of miraxanthin V as expressed by its peak area to the total chromatogram area at 280 nm exceeded 90%, indicating only traces of dopamine present. This standard solution was lyophilized, and the obtained powder was kept at −26 °C until further analysis.

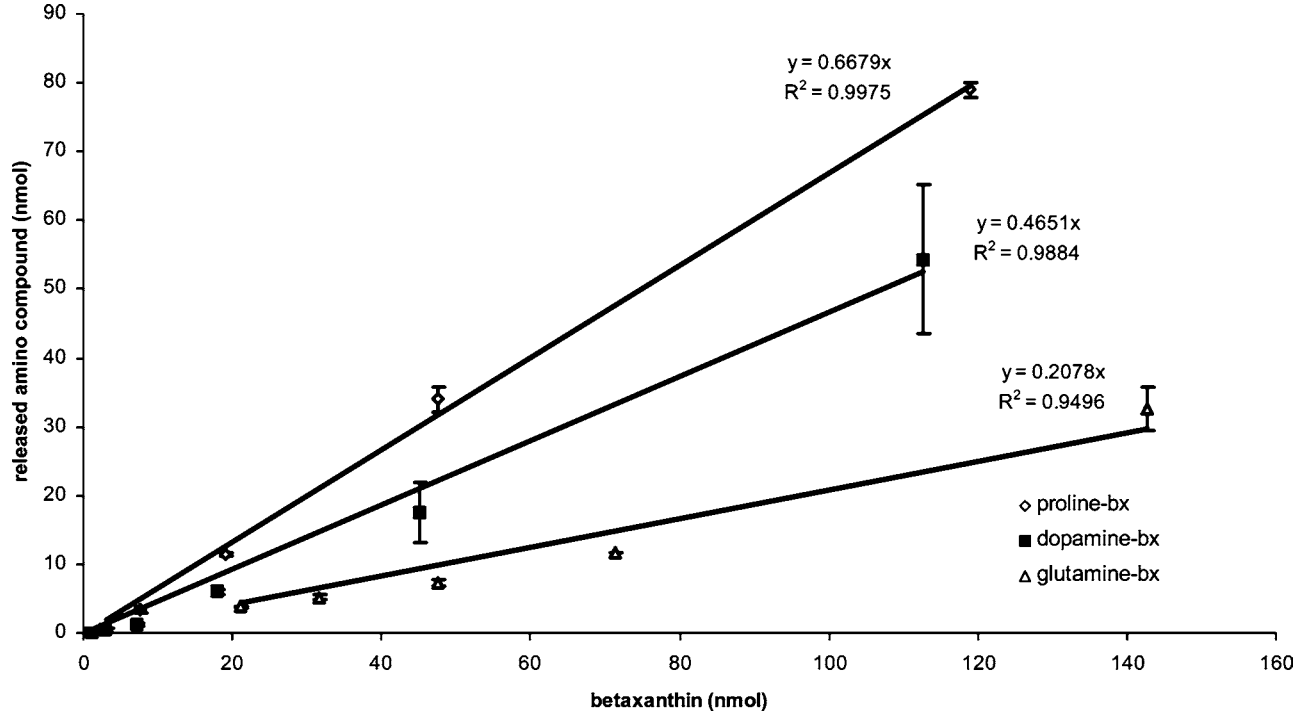

Figure 1. Concentration-dependent release of amino compounds from the corresponding bx upon derivatization.

Vulgaxanthin I. Isolation of vulgaxanthin I from yellow beet extract was carried out on an analytical HPLC system (Merck, Darmstadt, Germany) equipped with an autosampler L-7200, an interface module D-7000, a pump L-7100, a column oven L-7350 with a Peltier cooling module, and a UV−vis detector L-7400. An analytical scale (250 mm × 4.6 mm i.d.) Atlantis dC_{18} reversed phase column with a particle size of 5 μm (Waters, Wexford, Ireland), fitted with a C_{18}-ODS (4 mm × 3.0 mm i.d.) security guard column, was used for pigment isolation, operating at 20 °C and a flow rate of 1 mL/min. The mobile phase consisted of 1% formic acid in water (v/v, eluent A) and a mixture of MeCN in water of 80/20 (v/v, eluent B). Starting isocratically with 100% A for 2 min, a linear gradient from 0% B to 7% B in 21 min and from 7% B to 100% B in 5 min were followed before re-equilibration to starting conditions. Monitoring of vulgaxanthin I (R_t = 17.0 min) was performed at 470 nm. The collected eluates were immediately frozen at −26 °C, finally thawed, pooled, and concentrated in vacuo at 30 °C. The resulting residue was resuspended in 3 mL of purified water.

To exclude impurities due to native glutamine from yellow beet extract, fractions from R_t = 0 min to R_t = 12 min (fraction 1) and from R_t = 12 min to R_t = 16 min (fraction 2) were collected, concentrated at 30 °C under reduced pressure, and derivatized as described above. Glutamine could only be detected in fraction 1 and was consequently not present as a genuine amino acid in the collected vulgaxanthin I eluates.

Release of Amino Compounds from bx Standards during Sample Preparation and Derivatization. The concentrations of indicaxanthin, miraxanthin V, and vulgaxanthin I in the aqueous standard solutions were determined spectrophotometrically as described above except pH adaption with McIlvaine buffer. A 100 μL amount of diluted bx solutions was employed for sample preparation and derivatization and finally analyzed by GC-FID. The amount of the amino compounds released by alkaline hydrolysis was quantified in duplicate determinations.

RESULTS AND DISCUSSION

Appraisal of the "EZ:faast" Method for the Analysis of Betalainic Plant Material. Originally developed for clinical analyses, alkyl chloroformate derivatization has been successfully applied only recently for the characterization of amino acid profiles in various food samples such as honey, vegetables, and grains (*16*−*18*). Therefore, this rapid and sensitive method with a simple sample workup was considered suitable for a detailed screening of different fruit and vegetable samples from the cactus (Cactaceae) and goosefoot family (Chenopodiaceae). According to supplier's information, the "EZ:faast" method allows the separation and quantification of more than 50 amino compounds by GC after derivatization in alkaline solution containing sodium hydroxide and an organic base. To address the possible release of amino compounds by alkaline bx cleavage during sample preparation and derivatization, aqueous solutions of the isolated major bxs from different bx-containing plant materials were derivatized, namely, proline-bx from cactus pear (*19*), glutamine-bx from yellow beet (*19*), and dopamine-bx from Swiss chard (*12*), respectively. The released amino compounds were quantified by GC as shown in **Figure 1**. For each bx, a satisfactory linear relationship ($R^2 \geq 0.95$) was found. Different slopes of the regression lines may be either due to a varying affinity of bxs to the SPE material but also to a different sensitivity of the aldimine bond toward alkaline hydrolysis. By applying the regression functions obtained (**Figure 1**) and respecting these for the bx and amino compound contents in the investigated extracts, the contribution to the genuine amounts of the corresponding amino compounds was determined (see **Table 1**) and never exceeded 6.4%. Thus, pigment cleavage was found to be negligible for the bxs investigated. It should be considered that the quantitative deviation of the determined amino compound contents also depends on the

Table 1. Contribution of bx Cleavage (%) to the Genuine Content of Amino Compounds in Different Plant Materials Based on Individual bx Determination by HPLC-DAD Applying Equations from **Figure 1**

plant material	glutamine	proline	dopamine
yellow beet	0.60	2.99	6.36
yellow Swiss chard	0.37	1.34	2.19
yellow-orange cactus pear	0.15	0.71	*a*

[a] Dopamine-bx was not detected.

Table 2. Chromatographic (GC-FID) and Characteristic Mass Fragment Data (GC-MS) of Detected Amino Compound Derivatives

elution order	retention time (min)	amino compound	abbreviation	*m/z*
1	1.61	alanine	Ala	130, 88
2	1.74	glycine	Gly	102, 116
3	1.86	α-amino-*n*-butyric acid	Aaba	144, 102
4	2.00	valine	Val	116, 98, 158
5	2.15	norvaline	Nva	158, 116
6	2.25	leucine	Leu	172, 130
7	2.33	isoleucine	Ile	130,172
8	2.60	threonine	Thr	101
9	2.64	γ-amino-*n*-butyric acid	Gaba	130, 144, 172
10	2.64	serine	Ser	101, 203, 146
11	2.75	proline	Pro	156, 114
12	2.87	asparagine	Asn	69, 113, 155
13	3.57	aspartic acid	Asp	216, 130
14	3.62	methionine	Met	203, 101
15	4.02	glutamic acid	Glu	170, 142, 230
16	4.08	phenylalanine	Phe	147, 91, 206
17	4.43	α-aminoadipic acid	Aad	98, 244
18	4.88	glutamine	Gln	84, 187
19	5.37	ornithine	Orn	156, 70, 114
20	5.43	tyramine	Tym	120, 107
21	5.72	lysine	Lys	170, 128
22	5.97	histidine	His	282, 136, 168
23	6.08	3-methoxytyramine	3-Mtym	150, 137
24	6.33	tyrosine	Tyr	164, 206, 107
25	6.75	tryptophan	Trp	130
26	7.02	dopamine	Da	136, 123
27	7.65	3,4-dihydroxyphenylalanine	Dopa	222, 123

ratio of the bx to the respective amino compound. In addition, also, the matrix of derivatized extracts might influence the bindingof bxs to the SPE material and thus the amount of amino compound released.

After evaluation of the suitability of the "EZ:faast" method for analyzing betalainic food, 13 different plant materials were screened for 27 amino compounds. The mass fragment ions of derivatized amino standard compounds used for identification are listed in **Table 2**. In general, GC with MS detection was used for peak identification and quantification was performed by GC-FID using norvaline as an internal standard. As a compound structurally related to norvaline (*20*) and also the bx humilixanthin, the adduct of betalamic acid with hydroxynorvaline (*21*), have already been found in nature, genuinely present norvaline had to be excluded. GC-MS runs revealed that all investigated extracts were devoid of norvaline. A weakness of the method is the inability to detect derivatized arginine and citrulline (*18*, *22*), due to the adsorption of its ureic moiety to the GC column. Furthermore, γ-amino-*n*-butyric acid and serine could not be separated in this study, although different temperature programs were tested to improve peak resolution.

Cactus Pears and Pitayas. Juices from cactus pears and pitayas were subjected to amino compound profiling (**Table 3**). A similar pattern of free amino compounds was found for the juices from the cultivar Bianca with colorless pulp and the cultivar Rossa with red pulp. The high proline concentrations assumed to serve the plant as osmolyte to withstand drought (*23*) are in agreement with previous findings for fruits from the same and other *O. ficus-indica* cultivars (*14*, *24*). Moreover, comparatively high contents of aspartic and glutamic acids amounting to about 200 mg/kg juice were found for all cactus

Table 3. Amino Compounds[a] in Juices from Different Cactus Pear Cultivars and Pitaya Species (mg/kg of Juice) As Determined by GC-FID

	cactus pear cultivars			pitaya species		
	Bianca	Gialla	Rossa	*S. megalanthus*	*H. undatus*	*H. polyrhizus*
Ala	40.4 ± 0.1	24.9 ± 0.6	31.8 ± 0.3	5.2 ± 0.3	5.4 ± 0.1	4.8 ± 0.2
Gly	6.7 ± 0.1	3.6 ± 0.2	4.4 ± 0.1	2.8 ± 0.0	ND[b]	NQ[c]
Aaba	NQ[c]	NQ[c]	NQ[c]	ND	ND	ND
Val	35.5 ± 0.9	19.4 ± 0.6	24.1 ± 0.5	18.2 ± 0.5	1.6 ± 0.1	5.9 ± 0.2
Leu	18.9 ± 0.4	13.0 ± 0.3	17.8 ± 0.2	1.0 ± 0.1	NQ[c]	1.3 ± 0.0
Ile	31.9 ± 1.5	24.0 ± 1.3	29.1 ± 1.2	2.0 ± 0.2	NQ[c]	1.6 ± 0.1
Thr	8.9[d] ± 0.3	4.7[d] ± 0.4	8.2[d] ± 0.3	ND	NQ[c]	1.9 ± 0.0
Gaba[e]	418.2 ± 15.9	287.8 ± 13.6	321.8 ± 14.6	55.7 ± 3.7	7.1 ± 0.4	ND
Ser[e]	373.9 ± 15.3	248.8 ± 13.1	275.4 ± 14.0	49.1 ± 3.6	5.6 ± 0.4	17.4 ± 0.8
Pro	1411.3 ± 17.1	1476.9 ± 11.0	1837.2 ± 16.2	1803.7 ± 4.8	254.5 ± 4.1	273.3 ± 8.0
Asn	46.8 ± 0.6	29.5 ± 1.9	34.8 ± 0.8	18.5 ± 0.8	NQ[c]	1.1 ± 0.2
Asp	197.0 ± 24.4	188.7 ± 0.8	209.4 ± 5.3	80.7 ± 0.7	15.3 ± 0.1	95.0 ± 1.7
Met	NQ[f]	NQ[f]	NQ[f]	NQ[f]	NQ[f]	NQ[f]
Glu	171.3 ± 13.6	209.9 ± 10.1	215.5 ± 21.5	105.2 ± 7.7	97.7 ± 1.9	126.6 ± 12.4
Phe	18.1 ± 0.9	16.0 ± 0.3	22.8 ± 0.4	35.0 ± 0.6	4.8 ± 0.1	14.6 ± 0.2
Aad	NQ[c]	NQ[c]	NQ[c]	ND	ND	ND
Gln	205.7 ± 0.7	93.6 ± 7.8	116.5 ± 4.7	542.5 ± 9.0	11.3 ± 0.6	14.8 ± 0.2
Orn	ND	ND	ND	9.4	NQ[c]	3.3 ± 0.2
Tym	ND	ND	ND	NQ[f]	NQ[f]	NQ[f]
Lys	9.2 ± 0.6	7.9 ± 0.6	10.6 ± 0.4	3.6 ± 0.0	2.8 ± 0.0	5.1 ± 0.3
His	39.6 ± 0.7	34.0 ± 1.3	50.0 ± 1.8	18.1 ± 0.3	7.8 ± 0.1	11.9 ± 0.9
3-Mtym	ND	NQ[c]	NQ[c]	ND	ND	9.9 ± 0.7
Tyr	19.4 ± 0.5	19.3 ± 0.7	20.2 ± 0.6	9.7 ± 0.2	4.3 ± 0.0	6.9 ± 0.6
Trp	12.9 ± 0.1	13.0 ± 0.4	17.6 ± 0.6	19.0 ± 0.4	5.4 ± 0.0	8.6 ± 0.4
Da	ND	ND	ND	NQ[c]	4.0 ± 0.3	5.2 ± 0.7
Dopa	NQ[c]	NQ[c]	NQ[c]	NQ[c]	5.8 ± 0.1	5.4 ± 0.4
Σ[g]	2273.6	2178.4	2650.0	2674.6	420.7	597.2
bx[h]	*i*	50.7 ± 0.6	40.4 ± 0.1	*i*	*i*	*i*
bc[h]	*i*	4.9 ± 0.1	72.1 ± 0.4	*i*	*i*	553.4 ± 1.1
Σ[k]	*i*	55.6	112.5	*i*	*i*	553.4

[a] Means of triplicate determinations (± standard deviation). [b] ND, not detected; that is, no mass signals could be obtained. [c] NQ, not quantified; mass signals were detected. [d] The mass signal of Thr was not distinguishable from mass signal of Ser. [e] Because separation of Gaba and Ser was not achieved, the respective peak area was calculated as either Gaba or Ser. [f] Not quantified as a ghost peak coeluted; mass signals were detected. [g] Total amount of amino compounds excluding Gaba and Ser values. [h] bx (proline-bx equivalents) and betacyanin (bc; betanin equivalents) contents (mg/kg of juice) were obtained by photometric quantification (means of triplicate determinations ± standard deviation). [i] No betalains present. [k] Total betalain content (mg/kg of juice) obtained by summing bx and bc contents.

Table 4. Amino Compounds[a] in Colored Swiss Chard Petioles and Red and Yellow Beets (mg/kg of Fresh Weight) As Determined by GC-FID

	Swiss chard stems					beet roots	
	white	yellow	yellow-orange	red-purple	purple	yellow	red
Ala	47.0 ± 1.2	125.9 ± 1.7	43.9 ± 0.3	98.1 ± 1.9	75.2 ± 0.7	237.4 ± 5.6	147.5 ± 2.2
Gly	12.2 ± 0.6	28.0 ± 0.3	11.2 ± 0.1	33.9 ± 0.2	22.5 ± 0.2	48.5 ± 1.3	41.8 ± 0.6
Aaba	NQ[b]	NQ[b]	NQ[b]	NQ[b]	NQ[b]	NQ[b]	NQ[b]
Val	7.5 ± 0.1	16.8 ± 0.2	11.7 ± 0.2	22.9 ± 0.3	11.7 ± 0.0	88.6 ± 1.6	56.4 ± 0.4
Leu	8.8 ± 0.0	16.4 ± 0.1	13.9 ± 0.0	20.4 ± 0.1	13.9 ± 0.6	194.4 ± 1.7	112.7 ± 0.7
Ile	6.1 ± 0.2	14.7 ± 0.4	12.3 ± 0.6	26.9 ± 0.5	10.6 ± 0.8	200.1 ± 2.1	128.3 ± 2.1
Thr	8.0 ± 0.2	22.3 ± 0.4	13.7 ± 0.7	27.0 ± 1.7	15.7 ± 0.9	62.7 ± 0.3	63.3 ± 1.6
Gaba[c]	65.0 ± 1.9	158.1 ± 1.4	89.9 ± 2.1	159.1 ± 6.9	105.4 ± 4.2	334.1 ± 13.1	270.2 ± 11.3
Ser[c]	58.5 ± 1.9	141.5 ± 1.3	81.2 ± 2.0	141.1 ± 6.7	92.6 ± 4.1	315.0 ± 12.6	244.1 ± 10.9
Pro	3.7 ± 0.1	4.6 ± 0.1	4.2 ± 0.0	4.4 ± 0.0	4.6 ± 0.1	6.3 ± 0.3	6.1 ± 0.3
Asn	28.8 ± 1.3	92.7 ± 4.7	53.8 ± 4.5	102.1 ± 5.1	79.4 ± 2.1	410.6 ± 17.8	348.0 ± 9.2
Asp	134.3 ± 3.9	109.3 ± 0.7	117.7 ± 5.8	136.2 ± 3.5	130.9 ± 3.4	203.0 ± 5.8	285.1 ± 3.7
Met	NQ[d]	NQ[d]	NQ[d]	NQ[d]	NQ[d]	NQ[d]	NQ[d]
Glu	237.8 ± 18.4	313.4 ± 4.9	320.5 ± 21.4	300.5 ± 12.1	286.8 ± 15.9	635.0 ± 36.8	766.5 ± 25.2
Phe	ND[e]	ND	ND	ND	ND	16.9 ± 0.6	ND
Aad	NQ[b]	NQ[b]	NQ[b]	NQ[b]	NQ[b]	ND	ND
Gln	318.0 ± 5.8	1129.6 ± 66.6	643.6 ± 29.4	1224.7 ± 51.1	835.9 ± 32.9	2888.3 ± 75.2	2481.9 ± 92.4
Orn	ND	ND	ND	NQ[b]	NQ[b]	NQ[b]	9.0 ± 0.3
Tym	NQ[d]	NQ[d]	NQ[d]	NQ[d]	NQ[d]	NQ[d]	NQ[d]
Lys	8.9 ± 0.3	17.1 ± 1.1	14.1 ± 0.9	16.4 ± 1.1	14.6 ± 0.5	28.0 ± 1.4	32.9 ± 1.0
His	ND	10.8 ± 0.1	9.1 ± 0.3	13.8 ± 1.0	10.1 ± 0.1	41.5 ± 0.2	36.4 ± 0.3
3-Mtym	2.7 ± 0.9	5.2 ± 0.2	4.6 ± 0.7	3.1 ± 0.4	3.8 ± 0.2	5.8 ± 0.3	6.2 ± 0.5
Tyr	11.2 ± 0.2	21.3 ± 1.0	17.2 ± 0.4	32.0 ± 0.3	13.8 ± 0.1	125.5 ± 1.5	33.1 ± 1.0
Trp	7.6 ± 0.1	21.3 ± 0.1	16.5 ± 0.2	21.5 ± 0.5	14.8 ± 0.1	55.0 ± 1.0	40.9 ± 1.0
Da	88.4 ± 6.3	303.7 ± 21.3	255.9 ± 10.9	293.4 ± 51.1	190.9 ± 12.8	50.8 ± 3.5	366.3 ± 42.4
Dopa	8.8 ± 0.3	15.0 ± 0.3	9.1 ± 0.4	15.8 ± 2.6	20.8 ± 3.3	140.2 ± 2.4	27.4 ± 2.8
Σ[f]	939.8	2268.1	1573.0	2393.1	1756.0	5438.6	4989.8
bx[g]	*h*	107.4 ± 0.6	47.8 ± 0.3	64.7 ± 0.8	21.0 ± 0.1	297.3 ± 0.6	720.9 ± 2.1
bc[g]	*h*	*h*	22.6 ± 0.1	51.6 ± 0.8	45.8 ± 0.4	*h*	1245.1 ± 3.9
Σ[i]	*h*	107.4	70.4	116.3	66.8	297.3	1966.0

[a] Means of triplicate determinations (± standard deviation). [b] NQ, not quantified; mass signals were detected. [c] Because separation of Gaba and Ser was not achieved, the respective peak area was calculated as either Gaba or Ser. [d] Not quantified as a ghost peak coeluted; mass signals were detected. [e] ND, not detected; that is, no mass signals could be obtained. [f] Total amount of amino compounds excluding Gaba and Ser values. [g] bx (glutamine-bx equivalents) and betacyanin (bc; betanin equivalents) contents (mg/kg of fresh weight) were obtained by photometric quantification (means of triplicate determinations ± standard deviation). [h] No betalains present. [i] Total betalain content (mg/kg of fresh weight) obtained by summing up bx and bc contents.

pear cultivars. Besides arginine, taurine could not be detected with the applied "EZ:faast" method. Hence, high levels of taurine reported in previous investigations (*24*, *25*) were not detectable. In striking contrast to the *Opuntia* cultivars and the pitaya *S. megalanthus* (white pulp), both juices from *H. undatus* (white pulp) and *H. polyrhizus* (red-violet pulp) showed relatively low total amounts of free amino compounds. In accordance with a previous study (*14*), proline was the predominating free amino acid. Additionally, a high glutamine content (543 mg/kg) was detected in the juice from *S. megalanthus*. While the *Opuntia* cultivars Gialla and Rossa contain bxs with indicaxanthin as major compound (*14*, *19*), *H. polyrhizus* is devoid of yellow betalains and rich in betacyanins (*26*). Therefore, in the case of pitayas, the release of amino compounds by alkaline hydrolysis from bxs is not relevant.

Swiss Chard and Beet. In addition to Cactaceae fruits, extracts from Swiss chard and yellow and red beets (Chenopodiaceae) were included in this study. Their free amino compound contents are listed in **Table 4**, and an exemplary chromatogram for a yellow Swiss chard extract is shown in **Figure 2**. The lowest total amount in amino compounds was found in colorless Swiss chard stems. Generally, glutamine was the dominating component of Swiss chard stems, followed by glutamic and aspartic acids, respectively. Dopamine, earlier detected in fruits such as plums, avocados, and bananas (*27*) as well as in the leaves from beets and spinach (*28*), was found at high concentrations in Swiss chard petioles (up to 304 mg/kg fresh weight in yellow petioles) and in red beet roots (366 mg/kg), respectively. Thus, Swiss chard stems and beet roots contain even more dopamine than Cavendish banana pulp (25−100 mg/kg) (*29*). From a pharmacological view, dopamine is an important neurotransmitter in mammals. Because it is poorly absorbed and rapidly metabolized in the gut wall and the liver (*30*), respectively, it exerts no adverse effects on the human physiology when ingested. As already observed for tyramine (*28*), it can be assumed that concomitant administration of monoamine oxidase inhibitors and ingestion of dopamine-rich food might lead to circulatory disorders. On the other hand, dopamine is considered a strong water-soluble antioxidant and is supposed to act as phytochemical in preventing degenerative diseases (*29*). Interestingly, the bxs derived from the major amino acid glutamine and the main amine dopamine were found to be the dominating bxs in colored Swiss chard petioles (*12*) pointing to a positive quantitative correlation between the amino compound and the corresponding bx. The maximum values of free amino compounds of all plant materials investigated in this study were detected in red beet and the yellow cultivar Burpee's Golden. In accordance with Swiss chard stems, glutamine clearly dominated, followed by glutamic acid, asparagine, and aspartic acid. As described for colored Swiss chard (*12*), glutamine-bx was the major bx in extracts from yellow beet (*19*). Whereas 3,4-dihydroxyphenylalanine (Dopa), a key precursor in betalain biosynthesis (*31*), dominated over dopamine in yellow beet, an inverse relation was observed for red beet and Swiss chard petioles (**Table 4**). This might be ascribed to a higher L-tyrosine decarboxylase activity converting Dopa to dopamine in red beet (*32*). Food containing high concentrations of Dopa such as broad bean pods commonly consumed in New Zealand have been

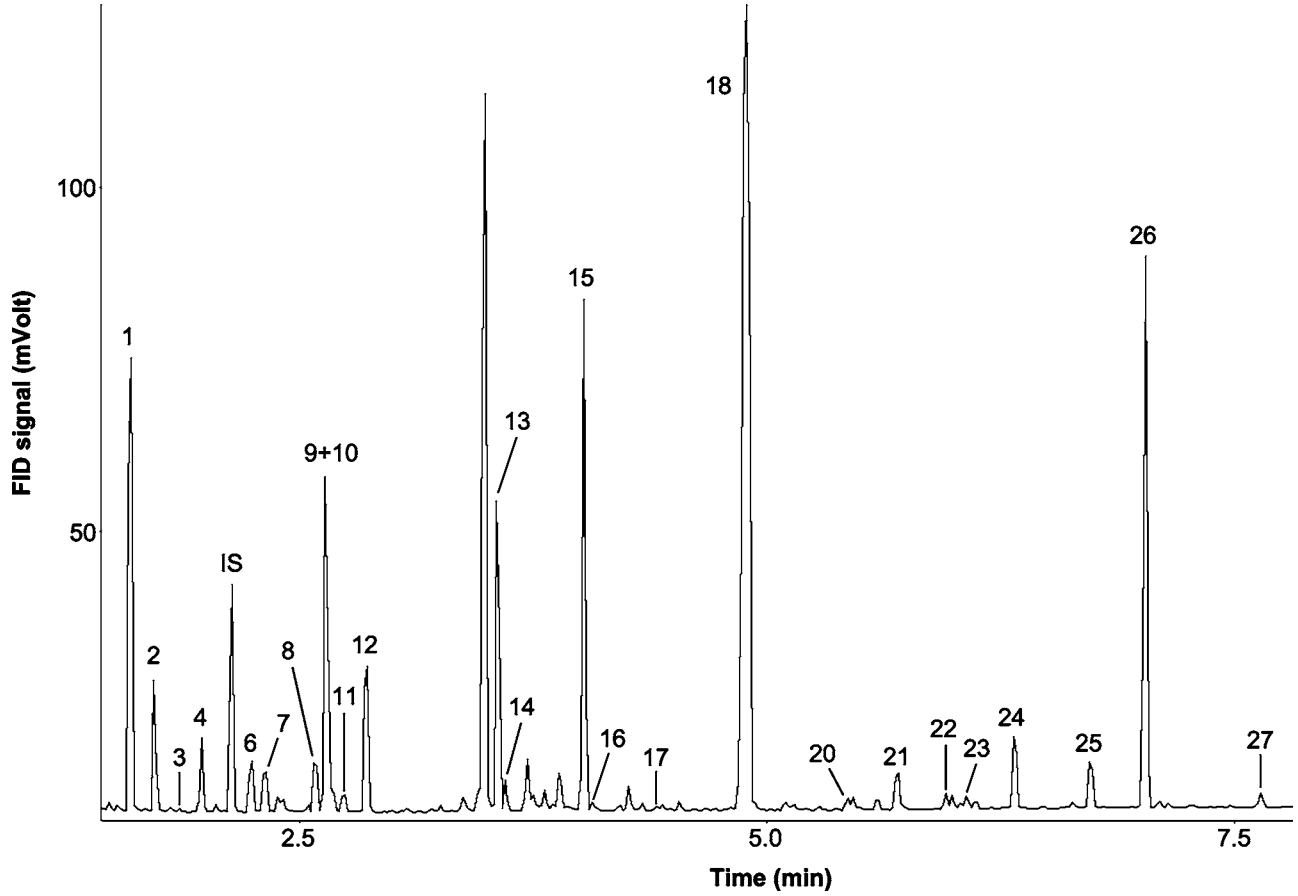

Figure 2. GC-FID fingerprint of amino compounds from an extract of yellow Swiss chard petioles (peak assignment in **Table 2**).

known to create a hypertensive crisis. The latter is considered attributable to the presence of Dopa, which may be converted to dopamine in the body (*28*). From a technological point of view, high contents of dopamine and Dopa may enhance browning reactions, thus adversely affecting the visual appearance of the resulting products.

In summary, the present study investigated the free amino compound profiles of potential betalainic coloring foodstuffs and colorless analogues, thus extending the knowledge on the composition of betalainic foodstuffs. To the best of our knowledge, a thorough investigation of the amino compound profile from pitaya fruits is presented for the first time. The determined amino compound profiles may provide useful information for the nutritive evaluation and appropriate technological processing of the respective plant materials but might also lead to a better understanding of betalain biosynthesis (*9*, *24*, *33*). Furthermore, never before scrutinized, the release of amino compounds following alkaline derivatization of bx-containing extracts has been demonstrated. This aspect should also be considered when handling extracts derived from plant materials containing other potential amino compound-releasing derivatives such as amino acid conjugates of jasmonic acid or phenolic amides (*34*, *35*), respectively.

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LITERATURE CITED

(1) Clement, J. S.; Mabry, T. J. Pigment evolution in the Caryophyllales: A systematic overview. *Bot. Acta* **1996**, *109*, 360−367.

(2) Michelot, D.; Melendez-Howell, L. M. *Amanita muscaria*: Chemistry, biology, toxicology, and ethnomycology. *Mycol. Res.* **2003**, *107*, 131−146.

(3) Stintzing, F. C.; Carle, R. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends Food Sci. Technol.* **2004**, *15*, 19−38.

(4) Kivi, J. T. Amino acids. In *Food Analysis by HPLC*, 2nd ed.; Nollet, L. M. L., Ed.; Marcel Dekker: New York, 2000; pp 80−84.

(5) Carratalá, M. L. M.; Moya, M. S. P.; Teruel, N. G.; Navarro, V. B. Discriminating significance of the free amino acid profile in almond seeds. *J. Agric. Food Chem.* **2002**, *50*, 6841−6846.

(6) Campanella, L.; Crescentini, G.; Avino, P. Simultaneous determination of cysteine, cystine and 18 other amino acids in various matrices by high-performance liquid chromatography. *J. Chromatogr. A* **1999**, *833*, 137−145.

(7) Cai, Y.; Sun, M.; Schliemann, W.; Corke, H. Chemical stability and colorant properties of betaxanthin pigments from *Celosia argentea*. *J. Agric. Food Chem.* **2001**, *49*, 4429−4435.

(8) Krause, I.; Bockhardt, A.; Neckermann, H.; Henle, T.; Klostermeyer, H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-performance liquid chromatography of the dabsyl derivatives. *J. Chromatogr. A* **1995**, *715*, 67−79.

(9) Böhm, H.; Mäck, G. Betaxanthin formation and free amino acids in hairy roots of *Beta vulgaris* var. *lutea* depending on nutrient medium and glutamate or glutamine feeding. *Phytochemistry* **2004**, *65*, 1361−1368.

(10) Van Wandelen, C.; Cohen, S. A. Using quaternary high-performance liquid chromatography eluent systems for separating 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate-derivatized amino acid mixtures. *J. Chromatogr. A* **1997**, *763*, 11−22.

(11) Steiner, U.; Schliemann, W.; Böhm, H.; Strack, D. Tyrosinase involved in betalain biosynthesis of higher plants. *Planta* **1999**, *208*, 114−124.

(12) Kugler, F.; Stintzing, F. C.; Carle, R. Identification of betalains from petioles of differently colored Swiss chard (*Beta vulgaris* L. ssp. *cicla* [L.] Alef. cv. Bright Lights) by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 2975−2981.

(13) Drawert, F.; Heimann, W.; Rolle, K. Über Aminosäuren in Früchten und deren Verhalten während Wachstum und Reife. I. Mitteilung: Quantitative Bestimmung von freien und gebundenen Aminosäuren in verschiedenen Früchten. *Z. Lebensm. Unters. Forsch.* **1970**, *144*, 237−245.

(14) Stintzing, F. C.; Schieber, A.; Carle, R. Evaluation of color properties and chemical quality parameters of cactus juices. *Eur. Food Res. Technol.* **2003**, *216*, 303−311.

(15) Chandra, A.; Rana, J.; Li, Y. Separation, identification, quantification, and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-MS. *J. Agric. Food Chem.* **2001**, *49*, 3515−3521.

(16) Nozal, M. J.; Bernal, J. L.; Toribio, M. L.; Diego, J. C.; Ruiz, A. Rapid and sensitive method for determining free amino acids in honey by gas chromatography with flame ionization or mass spectrometric detection. *J. Chromatogr. A* **2004**, *1047*, 137−146.

(17) Lee, J.; Harnly, J. M. Free amino acid and cysteine sulfoxide composition of 11 garlic (*Allium sativum* L.) cultivars by gas chromatography with flame ionization and mass selective detection. *J. Agric. Food Chem.* **2005**, *53*, 9100−9104.

(18) Elmore, J. S.; Koutsidis, G.; Dodson, A. T.; Mottram, D. S.; Wedzicha, B. L. Measurement of acrylamide and its precursors in potato, wheat, and rye model systems. *J. Agric. Food Chem.* **2005**, *53*, 1286−1293.

(19) Stintzing, F. C.; Schieber, A.; Carle, R. Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear [*Opuntia ficus-indica* (L.) Mill.] by high-performance liquid chromatography−electrospray ionization mass spectrometry. *J. Agric. Food Chem.* **2002**, *50*, 2302−2307.

(20) Levenberg, B. Isolation and characterization of beta-methylene-1-(+)-norvaline from *Lactarius helvus*. *J. Biol. Chem.* **1968**, *243*, 6009−6013.

(21) Strack, D.; Schmitt, D.; Reznik, H.; Boland, W.; Grotjahn, L.; Wray, V. Humilixanthin, a new betaxanthin from *Rivina humilis*. *Phytochemistry* **1987**, *26*, 2285−2287.

(22) Hušek, P.; Šimek, P. Advances in amino acid analysis. *LCGC* **2001**, *19*, 986−999.

(23) Stintzing, F. C.; Schieber, A.; Carle, R. Phytochemical and nutritional significance of cactus pear. *Eur. Food Res. Technol.* **2001**, *212*, 396−407.

(24) Stintzing, F. C.; Schieber, A.; Carle, R. Amino acid composition and betaxanthin formation in fruits from *Opuntia ficus-indica*. *Planta Med.* **1999**, *65*, 632−635.

(25) Tesoriere, L.; Fazzari, M.; Allegra, M.; Livrea, M. A. Biothiols, taurine, and lipid-soluble antioxidants in the edible pulp of Sicilian cactus pear (*Opuntia ficus-indica*) fruits and changes of bioactive juice components upon industrial processing. *J. Agric. Food Chem.* **2005**, *53*, 7851−7855.

(26) Stintzing, F. C.; Schieber, A.; Carle, R. Betacyanins in fruits from red-purple pitaya, *Hylocereus polyrhizus* (Weber) Britton & Rose. *Food Chem.* **2002**, *77*, 101−106, 517.

(27) Belitz, H.-D.; Grosch, W.; Schieberle, P. Obst und Obstprodukte. In *Lehrbuch der Lebensmittelchemie*, 5th ed.; Belitz, H.-D., Ed.; Springer-Verlag: Berlin, Heidelberg, New York, 2001; p 800.

(28) Smith, T. A. Amines in food. *Food Chem.* **1981**, *6*, 169−200.

(29) Kanazawa, K.; Sakakibara, H. High content of dopamine, a strong antioxidant, in Cavendish banana. *J. Agric. Food Chem.* **2000**, *48*, 844−848.

(30) Porzig, H.; Häusler, G. Pharmaka mit Wirkung auf das vegetative Nervensystem. In *Pharmakologie und Toxikologie. Lehrbuch für Studierende der Medizin, Pharmazie und Naturwissenschaften*, 5th ed.; Estler, C.-J., Ed.; Schattauer Verlag: Stuttgart, New York, 2000; p 82.

(31) Strack, D.; Vogt, T.; Schliemann, W. Recent advances in betalain research. *Phytochemistry* **2003**, *62*, 247−269.

(32) Facchini, P. J.; Huber-Allanach, K. L.; Tari, L. W. Plant aromatic L-amino acid decarboxylases: Evolution, biochemistry, regulation, and metabolic engineering applications. *Phytochemistry* **2000**, *54*, 121−138.

(33) Schliemann, W.; Kobayashi, N.; Strack, D. The decisive step in betaxanthin biosynthesis is a spontaneous reaction. *Plant Physiol.* **1999**, *119*, 1217−1232.

(34) Schmidt, J.; Kramell, R.; Schneider, G. Liquid chromatography/electrospray tandem mass spectrometry of amino acid conjugates of jasmonic acid under positive and negative ionisation. *Eur. Mass Spectrom.* **1995**, *1*, 573−581.

(35) Kujala, T. S.; Vienola, M. S.; Klika, K. D.; Loponen, J. M.; Pihlaja, K. Betalain and phenolic compositions of four beetroot (*Beta vulgaris*) cultivars. *Eur. Food Res. Technol.* **2002**, *214*, 505−510.

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